Establishment of a mouse model stably replicating and expressing hepatitis B virus genotype C prevailed in China

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This work was supported by the National Natural Science Foundation of China (81371852, 81573676, 81373136)  
\[Abstract\] Objective To establish a mouse model stably replicating and expressing hepatitis B virus genotype C (HBV-C) prevailed in China. Methods The recombinant adeno-associated virus rAAV8-1.3HBV-C (adr serotype) was transduced into human hepatocellular carcinoma HuH7 cells in vitro, and the expression of HBsAg and HBeAg in the cell culture supernatant was determined by ELISA. High expression recombinant virus rAAV8-1.3HBV-C was screened and injected via the tail vein into eight C57BL/6 mice (aged 6-8 weeks) as the experimental group; meanwhile the previously reported rAAV8-1.3HBV-D (ayw serotype) was injected into seven C57BL/6 mice as the control group. HBV DNA load, HBsAg and HBeAg levels in sera were assayed at weeks 2, 3, 5, 7, and 9 post viral injections. Results The supernatant HBsAg and HBeAg were detectable in the HuH7 cells 72h after transduction in vitro. The fluorescence quantitative PCR results of the HBV DNA load in serum at week 2, 3, 5, 7, and 9 post viral injection suggested stable in vivo replication of HBV DNA in mice. Conclusion The expression of HBeAg was stable while the serum expression of HBsAg fluctuated. No obvious inflammatory cell infiltration or abnormal structure of liver tissue was observed, while HBsAg and HBeAg expression in the...
liver tissue were detected for both groups. Conclusion By in vivo transduction with the recombinant virus rAAV8-1.3HBV-C, a mouse model that stably expressed and replicated HBV-C has been successfully established.

[Key words] hepatitis B virus; genotype; models, animal

1.2.1 HBV infection in vivo (ELISA) and HBeAg detection. The results showed that Huh7 cells were positive for HBeAg at 12 h post-infection, with an average concentration of 8 × 10^8 copies per cell. The concentration of HBeAg in the culture supernatant was measured by ELISA and found to be 24 pg/ml.

1.2.2 HBV replication in vitro. The results showed that the replication rate of HBV in the rAAV8-1.3HBV-D group was significantly higher than that in the rAAV8-1.3HBV-C group, with a replication efficiency of 84% in the former group.

1.2.3 HBV replication in vivo. The results showed that the replication rate of HBV in the rAAV8-1.3HBV-D group was significantly higher than that in the rAAV8-1.3HBV-C group, with a replication efficiency of 84% in the former group.

1.2.4 HBV replication in vivo (ELISA) and HBeAg detection. The results showed that Huh7 cells were positive for HBeAg at 12 h post-infection, with an average concentration of 8 × 10^8 copies per cell. The concentration of HBeAg in the culture supernatant was measured by ELISA and found to be 24 pg/ml.

1.2.5 HBV replication in vivo (ELISA) and HBeAg detection. The results showed that Huh7 cells were positive for HBeAg at 12 h post-infection, with an average concentration of 8 × 10^8 copies per cell. The concentration of HBeAg in the culture supernatant was measured by ELISA and found to be 24 pg/ml.
1.3 统计学处理 采用SPSS 20.0软件进行统计分析，数据结果以±s 表示，两组间均数的比较采用t检验，P<0.05为差异有统计学意义。

2 结果

2.1 重组病毒转导后HuH7细胞中HBsAg和HBeAg的表达变化 未转染重组病毒的细胞对照上清中未检测到HBsAg和HBeAg表达，而转导rAAV8-1.3HBV-C和AAV8-1.3HBV-D后HuH7细胞可有效表达HBsAg和HBeAg，且两者相比无明显差异(图1)。

2.2 模型小鼠血清HBV DNA载量检测结果 实验组和对照组小鼠第2、3、5、7、9周均能在血清中检测到HBV DNA且病毒载量相对稳定，但实验组血清DNA水平显著低于对照组(P<0.05，图2)。第9周末提取小鼠肝组织行HBV DNA定量，结果显示，实验组和对照组HBV DNA定量分别为8.08、0.20和8.38、0.11lg(拷贝/ml)，两组比较差异无统计学意义(P>0.05)。

2.3 模型小鼠血清抗原检测结果 C57BL/6小鼠注射rAAV8-1.3HBV病毒后的第2、3、5、7、9周，分别用ELISA法检测小鼠血清(1:10稀释)中HBsAg和HBeAg的水平，结果分别如图3A和图3B所示，注射病毒2周后实验组和对照组小鼠血清样本中均可检测到HBsAg和HBeAg持续高表达，且HBeAg的表达水平较HBsAg更平稳。

2.4 小鼠肝组织HE染色及免疫组化染色结果 取
图4 小鼠肝组织病理检测结果(100)

Hematoxylin and eosin (HE) staining of mice injected with rAAV8-1.3HBV-C and rAAV8-1.3HBV-D (A, D); Immunohistochemistry for HBsAg expression in the liver of mice injected with rAAV8-1.3HBV-C and rAAV8-1.3HBV-D (B, E); Immunohistochemistry for HBcAg in the liver of mice injected with rAAV8-1.3HBV-C and rAAV8-1.3HBV-D (C, F). Arrows show positive staining cells.


